<Division of Biochemistry>Molecular Biology

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– Molecular Biology –

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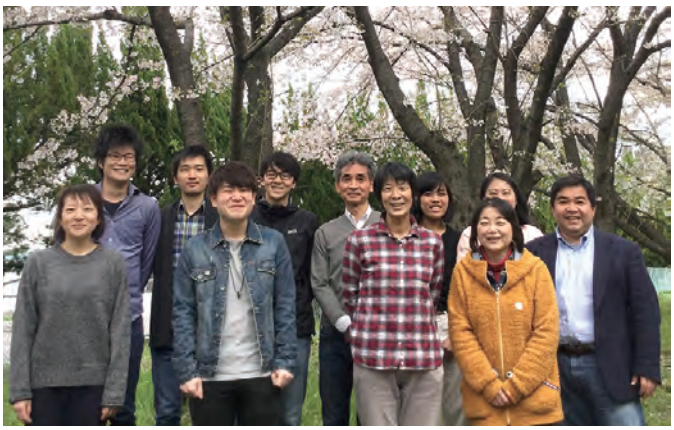
Scope of Research

This laboratory aims at clarifying molecular bases of regulatory mechanisms for plant development, especially plant morphogenesis, with techniques of forward and reverse genetics, molecular biology, and biochemistry. Current major subjects are: 1) phospholipid signaling in cell morphogenesis, 2) the transcriptional network for cytokinin responses, 3) COP9 signalosome modulating signal transduction in the nuclei, and 4) the endoreduplication cell cycle in cell differentiation.



KEYWORDS

Morphogenesis
Phospholipid Signaling
RNA
Signal Transduction
COP9 Signalosome

Selected Publications

Kato, M.; Tsuge, T.; Maeshima, M.; Aoyama, T., *Arabidopsis* PCaP2 Modulates the Phosphatidylinositol 4,5-bisphosphate Signal on the Plasma Membrane and Attenuates Root Hair Elongation, *Plant J.*, **99,** 610-625 (2019).

Lin, Q.; Ohashi, Y.; Kato, M.; Tsuge, T.; Gu, H.; Qu, L.-J.; Aoyama, T., GLABRA2 Directly Suppresses Basic Helix-loop-helix Transcription Factor Genes with Diverse Functions in Root Hair Development, *Plant Cell*, **27,** 2894-2906 (2015).

Wada, Y.; Kusano, H.; Tsuge, T.; Aoyama, T., Phosphatidylinositol Phosphate 5-kinase Genes Respond to Phosphate Deficiency for Root Hair Elongation in *Arabidopsis thaliana*, *Plant J.*, **81,** 426-437 (2015).

Hayashi, K.; Nakamura, S.; Fukunaga, S.; Nishimura, T.; Jenness, M. K.; Murphy, A. S.; Motose, H.; Nozaki, H.; Furutani, M.; Aoyama, T., Auxin Transport Sites are Visualized in Planta Using Fluorescent Auxin Analogs, *Proc. Natl. Acad. Sci. USA*, **111,** 11557-11562 (2014).

Kato, M.; Aoyama, T.; Maeshima, M., The Ca^{2+}-binding Protein PCaP2 Located on the Plasma Membrane is Involved in Root Hair Development as a Possible Signal Transducer, *Plant J.*, **74,** 690-700 (2013).

PCaP2 Modulates Phosphoinositide Signaling on the Plasma Membrane

Arabidopsis plasma membrane-associated Ca^{2+}-binding protein-2 (PCaP2), which belongs to a class of plant-unique Ca^{2+}-binding proteins, also binds to phosphoinositides including phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2]. PCaP2, together with PCaP1, was first characterized as a protein related to *Raphanus sativus* radish vacuole Ca^{2+}-binding protein (RVCaB), and was later found to be associated with the plasma membrane *via* the *N*-myristoyl anchor and to bind Ca^{2+}, the Ca^{2+}/calmodulin complex, and phosphoinositides. Detailed biochemical analysis of PCaP2 has revealed that the 23-amino acid N-terminal polybasic region (PCaP2^{N23}) contains the *N*-myristoylation site and the sites of Ca^{2+}/calmodulin- and phosphoinositide-binding activity, while the residual acidic region (PCaP2$^{\Delta 23}$) is the location of Ca^{2+}-binding activity. In histochemical analysis, the *PCaP2* promoter was preferentially active in root hairs and pollen tubes, both of which are cellular structures formed by tip growth. While *pcap2* knockdown mutant exhibited longer root hairs than the wild type, root hair cell-specific overexpression of PCaP2^{N23} led to short-root-hair or no-root-hair phenotypes, and this defect was suppressed by overexpression of PIP5K3-YFP. These findings suggest that PCaP2 negatively modulates root hair elongation *via* its PtdIns(4,5)P_2-binding activity.

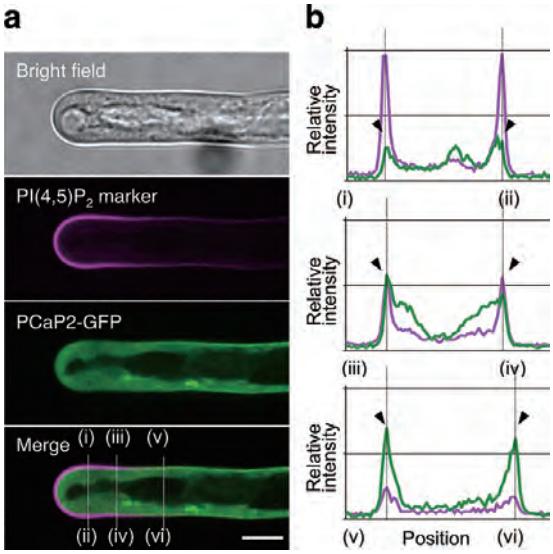


Figure 1. (a) Bright-field and fluorescence images (PCaP2-GFP and PI(4,5)P_2 marker) of elongating root hairs are shown. (b) The localization profiles of PCaP2-GFP (green) and the PI(4,5)P_2 marker (magenta) in the root hair apical region indicated in (a) are shown. In each column, a merged fluorescence image of PCaP2-GFP and the PI(4,5)P_2 marker, and their relative fluorescence intensities along the lines indicated in the image are shown. Dashed lines indicate the PI(4,5)P_2 marker localization on the plasma membrane. Arrowheads indicate the merge of PCaP2-GFP localization and PI(4,5)P_2 marker. Bars= 10 mm.

We focused on the function of PCaP2 *via* PtdIns(4,5)P_2-binding activity on the plasma membrane, and investigated the mechanism by which PCaP2 modulates root hair elongation. We found that the *pcap2* knockdown mutation caused a higher rate of root hair elongation than the wild type and partly suppressed the phenotype of a low elongation rate in the *pip5k3-2* mutant. Constitutively expressed 2xCHERRY-2xPH^{PLC}, a PtdIns(4,5)P_2 marker protein, and *PCaP2* promoter-driven PCaP2-GFP overlapped on the subapical plasma membrane of elongating root hairs (Figure 1). PCaP2^{N23}-GFP, which caused a low root hair elongation rate, exhibited a similar localization pattern on the plasma membrane to PCaP2-GFP. Inducibly overexpressed PCaP2-GFP, but not PCaP2$^{\Delta 23}$-GFP, replaced 2xCHERRY-2xPH^{PLC} on the plasma membrane in root meristematic epidermal cells (Figure 2), and suppressed FM4-64 internalization in elongating root hairs. Moreover, inducibly overexpressed PCaP2, but not PCaP2$^{\Delta 23}$, inhibited the endocytic recycling of PIN2-GFP, suggesting that PCaP2 affected the clathrin-mediated endocytosis involving the function of PtdIns(4,5)P_2 on the plasma membrane. Together, these results consistently support our idea that PCaP2 negatively modulates PtdIns(4,5)P_2 signaling on the subapical plasma membrane of elongating root hairs through competitive binding to PtdIns(4,5)P_2.

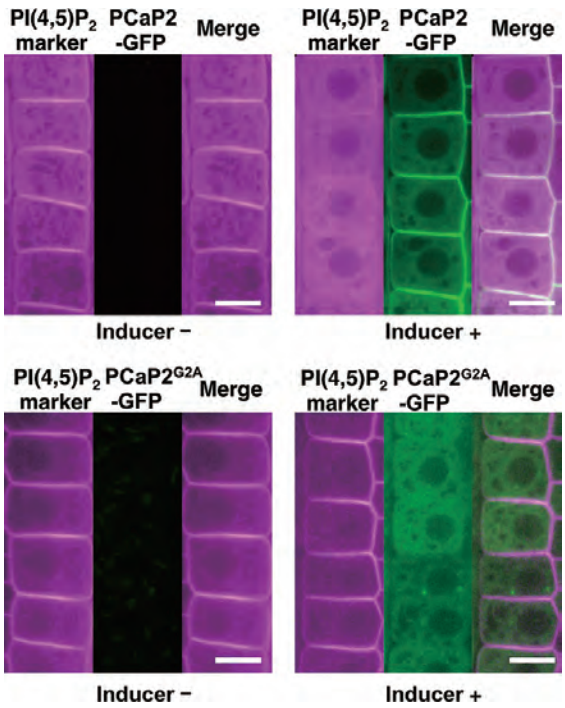


Figure 2. Fluorescence images of PCaP2-GFP (a) or PCaP2^{G2A}-GFP (b) expressed by an estradiol -inducible promoter, the PI(4,5)P_2 marker (magenta) expressed by the *UBIQUITIN10* promoter, and their merged images in meristematic root epidermal cells treated with (right panels) or without (left panels) 10 μM β-estradiol for 5 hours are shown. Bars = 10 mm.